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STORAGE

RED ROT OF SUGARCANE

BY

E. J. BUTLER, M.B., F.L.S

Imperial Mycologist

AND

ABDUL HAFIZ KHAN

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BY

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The most serious disease to which sugarcane is subject in India is undoubtedly that known as "red rot," caused by the fungus *Colletotrichum falcatum* Went. In 1906, a preliminary account of its characters and the damage caused by it in Bengal, especially that part which is now Bihar, was published in these Memoirs.[1] The most important conclusions there come to were that the disease ordinarily results in Northern India from the use of infected canes as "seed" and that the most hopeful method of checking it was by careful selection of the setts at the time of planting.

In a subsequent paper[2] the advantage of this practice was emphasised, some striking illustrations being given. Further experience has only increased the evidence of the value of sett selection which, while not an infallible preventive, is ordinarily instrumental in greatly diminishing the incidence of the disease. It is worth considering this evidence in detail, since infection from diseased seed has recently been denied by American[3] and West Indian[4] writers.

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[1] Butler, E. J. Fungus diseases of sugarcane in Bengal. Mem. Dept. of Agric. in India, Bot. Ser. I, No. 3, 1906.

[2] *Ib.* The selection of sugarcane cuttings. Agric. Journ. of India, II, 1907, p. 193.

[3] Edgerton, C. W. The red rot of sugarcane. Louisiana Agric. Exper. Sta. Bull. 133, 1911, p. 11.

[4] Red rot fungus and the sugarcane in the West Indies. Agricultural News, XII, Nos. 286-7-8, 1913.

Sett selection.

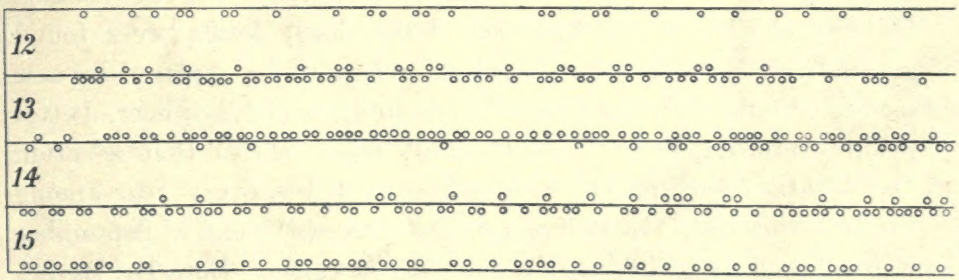
In 1906, alternate rows of diseased and healthy setts of a Madras cane, known as Yerra, were planted at Pusa, on February 26th-27th. Prior to planting, the presence of *Colletotrichum falcatum* had been determined in a large proportion of the canes from which the diseased setts were cut. By the 6th week after planting a decided difference was noticed in the two lots of seedlings. Those from diseased canes were withering in considerable numbers. *Colletotrichum falcatum* was found, in practically every case examined, in the young shoots, usually in the mycelial condition but in several instances producing spores at the basal nodes. In this, as in other cases where it was necessary to determine the identity of the organisms present in diseased cane, considerable use was made of the method of incubating aseptically removed slabs, described on page 8 of the Memoir above referred to. White ants, which are a frequent cause of injury to cane seedlings and which complicate the diagnosis in many cases of fungal attack, were absent from this particular crop, probably because the field in which it was grown was liable to flooding. *Sphæronema adiposum*, a fungus which is able to attack cut setts but not uninjured cane, and a parasite belonging to the genus *Cephalosporium*, which will be described in a subsequent paper, were found in a few cases.

The field in which this crop was grown had not been under cane in recent years and no other cane had been grown the previous year within about half a mile. There was, therefore, no reasonable risk of external infection and certainly no possibility of such an infection as would lead to the death of many plants in the trenches planted with setts from diseased cane, while the alternate, strictly comparable, trenches with healthy seed escaped. The photograph reproduced as Plate XII in the Agricultural Journal of India for 1907, shows the appearance of this plot on May 17th, and could hardly be more decisive. Under the microscope it was easy to trace the fungus from the setts up into the young shoots, and throughout April and May continued infection of the young shoots from their point of

origin occurred. On May 30th the number of sound shoots was counted in the trenches numbered 3 to 18, of which the odd numbers were from healthy seed and the even from diseased, the result being 679 and 117 respectively. The crop was lost by a severe flood in August, so that the final result cannot be given.

In 1907, the experiment was repeated in a field not subject to flooding, ten trenches being sown on March 7th with the varieties Striped Mauritius and Red Mauritius. By the end of April the results were as striking as in the previous year, but in May many shoots withered in the trenches planted with healthy seed of the Red Mauritius variety. This variety was taken from a diseased field and was so generally infected that the ratoons nearly all died out. It is probable that many of the apparently healthy canes contained *Colletotrichum*, for, as will be shown below, while reddening of the pith is a sure indication of disease, unless the canes have been mechanically injured, absence of reddening does not always imply freedom from it.

In 1908, the cane selected was again Red Mauritius, which was planted on March 6th. Germination was good in all the trenches. The condition of four rows, Nos. 12 to 15, on May 30th, is graphically shown below, the small circles each representing a sound shoot. Rows 12 and 14 were from diseased seed, rows 13 and 15 from healthy.



Besides these comparative experiments, the main crop of cane grown on the Pusa Farm has been yearly supervised, so that setts showing red marks in the pith are not planted. The result has been that, excepting the season 1907-08, which will be separately con-

sidered as the cane was weakened by an attack of sugarcane-fly, no serious outbreak of red rot has occurred since 1905-06. The disease is always present and was fairly bad in Yerra in 1905-06 and in Red Mauritius in 1906-07, in both cases accumulating in the following 1st ratoons so as to destroy most of the crop. In 1908-09 it was difficult to find enough diseased cane to supply cultures for experimental work. In other years we have had 5 to 10% diseased. These results are probably better than in any other estate in Bihar, though recently several estates have adopted sett selection. In one such case, that referred to on page 198 of the Agricultural Journal of India for 1907, a very severe attack of red rot occurred in 1905. Sett selection has since been carried out as a routine practice and the Manager reported recently that he has now no disease in his crop.

At the Samalkota Sugar Station in Madras, sett selection has been regularly carried out for the past ten years, at the instance of Dr. C. A. Barber, now Government Sugarcane Expert, Madras. Dr. Barber was, we believe, the first to advocate this method of fighting red rot.[1] In the beginning it was combined with pickling the setts in a strong mixture of lime water and carbolic, with a view to checking moth-borer and the Queensland Acarus "rust", but this was subsequently not considered necessary. In 1906, Dr. Barber stated that it was not possible by the mere rejection of red-marked setts to root out the disease[2]. In spite of all care all but two of the local kinds were found gradually to become worse, until they had to be replaced by new seed from outside. Once these had been discarded, however, better results were obtained. Thus the 1907 report states that selection had a satisfactory effect, disease becoming less every year among the best varieties. In 1908, a severe storm at the end of September was followed by withering of a good many canes. Both Dr. Barber and the senior writer saw the crop in the following February and

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1 Sugarcane in the Godavari and Ganjam Districts. Dept. of Land Records and Agriculture, Madras, Bull., Vol. II, No. 43, 1901, p. 188.

2 Barber, C. A. Scientific Report of the Samalkota Agricultural Station for the year ending 31st March, 1906, p. 25.

agreed in considering it remarkably free from red rot, and in holding the injury to the roots caused by the storm responsible for most of the damage. We also found evidence of the existence of a root disease not previously known, which will be described in a subsequent paper. In 1909-10, red rot was still present on the farm but to a far less extent than heretofore. In 1910-11, very little disease was noted either on the farm or in the nursery, though it was still fairly prevalent outside the farm. In 1911-12, red rot was present only to a small extent, though found in nearly all the varieties. From these records it is clear that red rot has not been stamped out by sett selection. It is equally clear that the disease is much less prevalent than in the early years of the existence of the farm. The farm was started as a result of a disastrous outbreak of red rot in the Godavari Delta, which threatened the extinction of cane cultivation in that area. It is fair to assume that the local varieties, which were at first grown, were very largely from diseased stock. So long as these varieties were retained, sett selection did not give satisfactory results. When they were replaced by other, comparatively healthy varieties, sett selection was effective in keeping the disease under control.

It is now necessary to consider why sett selection has proved ineffective in checking disease when the seed was taken from a severely diseased crop.

In selecting healthy setts for planting under field conditions reliance must be placed on the absence of obvious reddening of the pith, visible at the cut ends. Disease was severe in the Pusa crop of 1907-08 and the opportunity was taken of testing how far this method could be relied on. On November 21st, 1907, 6 canes were selected which, on cutting into lengths, were found to have reddened internodes above and below, but in the middle to be free from obvious reddening. In 3 of these, careful examination with a lens revealed one or two fine reddened points, corresponding with the cut ends of small bundles; the other 3 appeared quite free from discoloration. Slabs were cut out aseptically and incubated, and the presence of *Colletotrichum falcatum* was demonstrated in one of the six. On

March 17th, 1908, the experiment was repeated with 24 canes slightly reddened at the base but apparently clean higher up. Slabs were cut from above the limit of the discoloration. Of these 15 showed no marks even with a lens, while 9 had minute red points. The presence of *Colletotrichum* was demonstrated in 3 of the former and 1 of the latter. Therefore, in an attack of the severity described, nearly 17 per cent. of apparently clean slabs, taken from canes slightly affected with red rot, were shown to contain the fungus within their tissues. Under such circumstances it would have been necessary to discard all canes, any part of which was discoloured.; new infections were occurring right up to harvest time and some would probably have escaped even such rigorous selection. It would be cheaper and more satisfactory under ordinary estate conditions to discard the whole crop and import healthy seed. In Pusa, since the amount of seed required was small and questions of cost and trouble did not arise, it was found possible to retain some of the more valuable varieties and the 1908-09 crop had little disease. The amount discarded was, however, very great and would have been ruinous under estate conditions.

All that can safely be stated, so far, is that planting setts from obviously diseased canes leads to a considerable development of disease in the resulting crop and that, provided the variety is fairly free from disease to start with, sett selection keeps red rot within reasonable limits, unless some untoward circumstance, such as the epidemic of cane-fly at Pusa in 1907-08, intervenes.

The infection of sound setts.

If there were no other method of perpetuating red rot than by the use of diseased seed, one could, of course, stamp it out, even in view of the above facts. This brings us to the consideration of the parasitism of *Colletotrichum falcatum* and of the means at its disposal for obtaining an entry into previously healthy cane. We can then more readily discuss the further measures for its control and the influence of external conditions, such as the attack of cane-fly at Pusa in 1907-08, on the prevalence of the disease.

As was pointed out formerly,[1] this fungus is not, in Northern India, provided with as suitable a mechanism for spore distribution as in the case with most parasitic fungi. As long as the cane is growing, there is comparatively little risk of air-borne contamination from the stems of diseased plants in the crop. Dead and rotting canes are, however, frequently well provided with spores. Enormous numbers are often found in the pith cavities of old canes. These may contaminate the soil or get into the irrigation water. They may thus reach the newly planted setts. Several experiments have been carried out to ascertain whether infection of sound setts may take place in this manner.

In 1908, a short trench of Purple Mauritius cane was planted on March 7th, the setts in one-half being previously dipped into a suspension of *Colletotrichum* spores, from a pure culture, in distilled water. On April 30th there were 13 living shoots belonging to 12 setts in the non-inoculated half and 6 belonging to 6 setts in the inoculated.

Similar experiments on a larger scale, in 1909, gave conflicting results, both inoculated and control canes showing a number of withered shoots after two months. On May 14th there were 167 healthy shoots and 27 withered in the control trench and 174 healthy and 40 withered in the inoculated. White ants were bad in both, but there was also evidence that *Colletotrichum* had reached the control trench, probably on the feet of the farm labourers, who walked up and down from trench to trench during the irrigation of the crop.

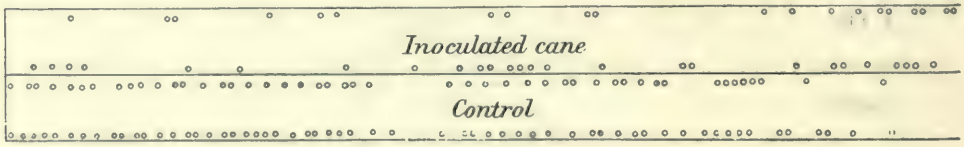
In 1910, the experiment of the previous year was repeated, the cane being sown on March 12th. Germination was slightly better in the control than in the inoculated trench and on May 3rd there were 325 shoots in the former and 304 in the latter. The control trench, which immediately adjoined the inoculated one, developed red rot as in the previous year, probably from infection during irrigation. A similar trench some 30 yards away, but supplied by a separate distributary, was, therefore, also selected for comparison. On June 21st there were only 255 healthy shoots left in the

[1] Butler, E. J., *loc. cit.*, 1906, p. 16.

inoculated trench, while there were 340 in the control trench and 784 in the trench further away.

A form of *Colletotrichum falcatum* which is truly parasitic on the leaves of sugarcane, was described and figured on page 13 and plate III, fig. 9 of the previous Memoir, and has also been found in Louisiana.[1] The ability of this form to cause typical red rot when introduced through setts has been demonstrated, as the following experiments show. As these experiments have a definite bearing on the question of the propagation of the disease through the setts they are included here, though the leaf form of the fungus will be more fully considered below.

On December 5th, 1907, 21 canes of Red Mauritius were inoculated from a pure culture of the leaf parasite shaken up in distilled water. The inoculations were done by removing a cylinder of cane at a lower internode with a small sterile cork borer, inserting some of the culture in the wound and replacing the cylinder after cutting a piece off the end with a flamed knife, so as to leave a cavity. The stem was then bound with fine sterile gutta-percha sheeting. On March 6th, 1908, the inoculated canes were cut and examined. One had been damaged by a jackal and was discarded. The others were outwardly sound. With 18 of these a trench was planted on March 7th, the canes being cut into the usual setts each with three "eyes." In cutting it was observed that obvious reddening of the pith occurred in from 1 to 3 internodes above the seat of inoculation. The remaining two canes were examined microscopically and the presence of *Colletotrichum* demonstrated. A similar trench was planted alongside, with sound setts from the same plot, to serve as a control. The condition on May 30th is shown graphically below. Red rot was severe in the inoculated trench and practically absent in the other.



[1] Edgerton, C. W., *loc. cit.*, p. 4, Pl. I.

On March 7th, 1908, a short trench of Purple Mauritius was planted, half with setts dipped in a pure suspension of the leaf form of *Colletotrichum*, the other half not inoculated. On April 30th, 62 healthy shoots belonging to 50 setts were found in the latter and 30 belonging to 26 setts in the former. Germination had been approximately equal in the two halves.

On March 12th, 1910, half a trench was similarly planted with setts of Ashy Mauritius, dipped in a pure suspension of the leaf form of *Colletotrichum*. Germination was better in the inoculated than in the control half, and on May 3rd there were 182 shoots in the former and 130 in the latter. On June 21st there were 77 healthy and 80 withering shoots in the inoculated half-trench, while the control half-trench had 156 healthy and 55 withering. As already stated the controls this year developed red rot, probably from infection during irrigation. A full trench of the same cane near by but supplied by a separate distributary had, as above mentioned, on this date 784 healthy shoots.

In all these cases the presence of *Colletotrichum* was demonstrated in several of the withering shoots, and they establish fully that true red rot can arise from infection from both the stem and the leaf forms of the fungus through the planted setts. Not only is the disease perpetuated by planting previously diseased setts, but healthy setts can be infected at the time of planting, if reached by the fungus, and, no doubt, subsequent infection from below ground can also occur. It is well known from previous work that the fungus can enter at wounds exposing the pith, such as the cut ends of the setts, and, as will be shown below, infection through the roots also readily occurs. The course of the infection up into the stem can be traced in many cases and direct connection between the mycelium in the sett and that in the new shoot established. Raciborski[1] has very correctly described the passage of the disease from the planted sett up into the young shoot.

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[1] Raciborski, M. De Bestrijding van het rood-snot. Archief v.d., Java-Suikerindustrie, V, 1897, p. 1133.

The infection of growing canes.

It is generally stated by workers outside India that red rot frequently arises from wound infection of the stem of the cane, after it has developed far enough to be exposed to the attacks of stem borer, that is usually in the second half of its growth period. Some observers even hold that this is the only way in which the disease can arise. The results of numerous inoculations, indeed, fully prove that cane can be artificially infected through wounds similar to those caused by insects. But, as was definitely stated by Prinsen Geerligs[1] in 1898, wound infection will not sufficiently explain every case of attack and we hope to show that in Northern India it is of secondary importance.

Went, who first described the disease, obtained successful inoculations by puncturing the rind with a fine needle and inserting conidia of *Colletotrichum*.[2] The infection was, however, localised and after 20 days was chiefly confined to the inoculated internode, traces only being found in the two higher up. Attempts to inoculate the unwounded rind failed, except when very young internodes were selected. Went concludes that natural infection occurs chiefly through the holes made by boring insects, but that the place of insertion of the leaf sheath at the node is also permeable. Howard,[3] ten years later, described the results of inoculations with the same fungus in the West Indies. When wound inoculations were made on vigorously growing canes about 6 months old, the fungus was found to have infected one or two internodes only, after two months. In fully grown cane, during the ripening period, however, infection was much more complete, up to 18 inches of the pith being invaded in less than a month, in one series. Inoculations at the leaf bases were successful in some cases but failed in others. Lewton-Brain[4] did a limited number of inoculations in Hawaii in

[1] Archief, VI, 1898, p. 450.

[2] Went, F. A. F. C. Het rood-snot. Mededeelingen Proefstation " West-Java," Archief v. d. Java-Suikerindustrie, I, 1893, p. 265.

[3] Howard, A. On some diseases of the sugarcane in the West Indies. Ann. of Bot. XVII, 1903, p. 373.

[4] Lewton-Brain, L. Red Rot of the Sugarcane stem. Exper. Sta. of the Hawaiian Sugar Planters' Assoc., Div. of Pathology and Physiology, Bull. 8, 1908.

1906-07. Wound inoculations on the stem of Yellow Caledonia (White Tanna) canes were made. After two months the inoculated internode was found to be infected and there appeared to be indications that the disease was spreading through the nodes to the internodes above and below. Ten months later, no further progress had been made. Presumably the inoculations were made on young plants. Infection through borer holes is considered by this writer to be practically the only way in which fresh attacks arise, but the propagation of the disease by planting diseased setts is accepted. Edgerton[1] reports a very large series of inoculations in Louisiana. He states that the disease spreads from the point of inoculation up and down through the cane for from two to five joints during the season, but is not visible externally. Sometimes, however, if the stalk is inoculated very young, the growth of the fungus is so rapid that the whole stalk is killed, but this is not usually the case. Infection through borer burrows is stated to be by far the commonest cause of the disease. Infection through the leaf bases and the rootlet buds at the nodes is considered possible but was not proven. Infection through the planted setts is denied. Selection of setts is advocated, not because they can carry red rot but because the resulting crop should be superior if only healthy seed is used.

Quite recently the results of inoculation experiments by South and Dunlop in St. Kitts and Barbados, are described in the " Agricultural News " (Vol. XII, Nos. 286-7-8, 1913). In the St. Kitts experiments wound inoculations at the nodes and internodes caused limited infection, which ceased to develop after about the first month. In the more susceptible canes the fungus spread quickly throughout the entire internode, but did not penetrate the joints. The cane was strongly growing White Transparent, seven months old. Inoculations on the leaf scars and between the leaf sheaths and the stem failed. Attempts to infect cuttings gave more complicated results. All the inoculated cuttings were reddened throughout after 83 days, while only about half the controls showed reddening

[1] Edgerton, C. W., *loc. cit.*, p. 5.

in every node and this was less intense as a rule. The latter cuttings had generally a white strand along the side from which the shoots arose. Of 30 inoculated cuttings 8 growing points were found to become diseased; a similar condition was observed in two controls. In one inoculated shoot *Colletotrichum* was identified. The control shoots were more numerous, weighed more and were generally more healthy in appearance than those from inoculated cuttings. In Barbados, 40 setts each of White Transparent, Bourbon, B 147 and B 376 were inoculated before planting, a parallel set being grown as controls. The inoculations took, but growth within the setts was slight, the discoloration being only visible for about 2 by $\frac{1}{2}$ inch round the wound after 6 weeks. There was no definite sign of penetration into the buds at this period. After three months there were 7 withered shoots in the controls and 15 in the inoculated cane. *Colletotrichum* could not be found in the diseased growing tips. The healthy shoots were cut off from the inoculated setts by the formation of a woody partition. The authors conclude that the fungus is a facultative wound parasite, infecting chiefly through wounds such as borer holes, and not carried into the young shoots from infected setts.

Since it is fully established that stem wounds allow of artificial infection, experiments were carried out with a view to ascertain how far this is a common origin of the disease in nature. Practically the only wounds which open a passage direct to the pith are those due to the various stem borers so commonly found in cane. These wounds were tested for *Colletotrichum* on several occasions.

In November, 1907, when red rot was prevalent at Pusa, six canes with borer holes were examined by cutting out aseptically a slab to include the reddened area always found in the immediate vicinity of the hole. On incubation none gave *Colletotrichum*. In the same month the following year the experiment was repeated with 9 bored canes. The result was again negative. In January, 1909, 12 bored canes were similarly examined at Samalkota, with the same result. In addition we have examined by sectioning the neighbourhood of the holes in bored canes on many occasions when the

crop was suffering more or less severely from red rot and have not been able to satisfy ourselves in any case that the disease had originated at the borer hole and not elsewhere. Yet we are satisfied that there are other methods of infection than from below ground and these may now be considered.

Went and Howard both refer to infection through the scar left when the leaf sheaths break away from the stem. In December, 1907, 20 Red Mauritius canes, almost fully grown, were inoculated at the scar left by pulling off a leaf about the centre of the stem. The leaves were old and came away readily. The inoculated portion was kept moist for about 24 hours by covering with moist sterile cotton wool. After three months the canes were examined. One was damaged by a jackal and was discarded. In 3, acervuli with spores of *Colletotrichum falcatum* were found on the surface of the scar. In 11, there was no reddening of the tissues and no sign of penetration. In 5, there was slight reddening at or near the node. No hyphæ, however, could be found and on incubating slabs, cut so as to include the reddened parts, no *Colletotrichum* developed. Even in the 3 cases where the fungus had fructified on the spot, no penetration occurred.

At the cane node there are two other points of discontinuity in the rind, where the shoot bud (the "eye") comes through and where the eyes of the adventitious roots occur. These were found to admit the fungus readily. In April, 1912, 12 buds of Samsara cane were injured by rubbing with the fingers and inoculated with spores and mycelium kept moist by cotton wool as before. One was examined after three days and the hyphæ were found to have penetrated the bud and to be growing vigorously. After eleven days another was examined. The mycelium was still confined to the bud, which was much reddened. A week later the rest were examined. All had taken the infection well and in several the hyphæ had already entered the main stem. Injury to the eye buds, especially as the cane approaches maturity, is unfortunately only too common, and the fungus can readily penetrate if such buds become contaminated. The uninjured

bud is less readily infected. Inoculations made by placing on the bud scales, in a moist atmosphere, fresh acervuli with spores, from a pure culture, showed little progress at the end of a month. There was a slight reddening of the scale, especially along the margin and hyphæ were numerous in the reddened part. The underlying bud layers were only faintly discoloured and very few hyphæ had entered them. The deeper layers of the bud were quite free. The buds were swelling at the time of inoculation and the progress of the infection was so extremely slow that it is doubtful if the young shoot could be reached before the outer layers had withered or lost contact with it. This is in harmony with our general experience that uninjured young shoots are not found attacked by red rot, unless the parent stem is also infected.

The adventitious root eyes are much more easily infected. Twelve Samsara canes were inoculated, in the same manner as in the last experiment, on April 16th, 1912, the root origins being prominent but quite sound and uninjured. On the 24th, 9 were examined and were all found infected, the hyphæ being well established and growing in towards the pith of the main stem. The experiment was repeated on May 22nd, 1913. Four perfectly healthy canes of a thin variety which had a good development of young, clean, adventitious roots, varying from about one-eighth of an inch to one inch in length, were inoculated in the laboratory in the manner described above. The culture used was five days old and 16 roots in all were inoculated. One root was sectioned after a week and found to have taken infection well. The penetration of the hyphæ into the tissues was clearly visible and is shown in fig. 3. Reddening had extended down the root and penetrated about $1\frac{1}{2}$ mm. into the main stem. Characteristic hyphæ of *Colletotrichum* were found in the reddened part of the stem. Two days later 3 more roots were examined, and the same conditions found. On this day the rind at the base of the root was found slightly discoloured and the discoloration extended during the following days, until it was clearly visible externally in all the inoculations. The normal dark green colour of the rind changed to a dirty mottled red, which spread

in vertically elongated streaks and, at the end of the 2nd week, entirely surrounded the node and had extended for an inch or two above and below. After twenty days several inoculated roots were examined at their origin from the stem and large quantities of hyphæ were found passing from the root to the stem, not only along the vascular tissues but also laterally into the stem parenchyma in all directions. Fig. 5 shows the conditions at this stage. The stems were split longitudinally a month after the inoculations and were found entirely infected, the characteristic pith discoloration, with transversely elongated white blotches, being well developed.

Further inoculations were made on June 3rd, 1913, on the feeding roots of well-established cane plants growing in large culture pots in soil. The soil was carefully removed until the roots were exposed and these were inoculated by sprinkling with a suspension of spores from a pure culture, care being taken not to injure the roots. The soil was then replaced. After sixteen days two of the inoculated roots were examined by sectioning. Both showed a small area of reddening 2 or 3 mm. behind the growing point. Penetration was found to have occurred here (Fig. 4) and the hyphæ were extending freely in the tissues of the root.

Out of many hundreds of canes affected with red rot examined during the past ten years, we have met with a limited number of cases where natural infection had occurred through some part of the stem above ground and where the base of the cane was unaffected. In February, 1910, a Khari cane was examined and was found to show definite symptoms of red rot in the 5th and 6th internodes from the base, the lowest internodes being quite free from reddening or hyphæ. At the node between the two infected internodes there was a broken shoot, distinctly reddened in its interior. Characteristic hyphæ of *Colletotrichum* were found in this shoot and could be traced from the broken surface through the bundles into the pith of the stem. There were no other injuries, and infection had doubtless occurred through the broken shoot. A second cane of the same variety showed a similar case of infection through a broken shoot at the 5th node. Higher up several nodes showed slight infection,

which was traced in every case to an infected adventitious rootlet eye. At the 20th node there was another broken shoot, through which infection had occurred. In all these cases, sections taken so as to include the shoot or root and the main stem, enabled the hyphæ to be clearly traced from one to the other. In another case infection occurred through root eyes at the 15th node and also in the 8th internode through a crack in the rind. This is one of the few cases where infection has been found arising from a definite stem wound. The cane was also affected with smut (*Ustilago Sacchari*).

From the above experiments it appears that wounds caused by boring insects, while undoubtedly capable of admitting the parasite should it reach them, are not, in practice, responsible for many cases of red rot in India. Old leaf scars are not readily penetrated, but since infection through the leaf bases has been obtained by Howard, this probably depends on the degree to which abscission has progressed at the time of inoculation. Under normal conditions the leaf scars are not exposed until the leaf has completely withered and, as our inoculations show, such scars are not readily infected. During the process of wrapping, which is common in parts of India, less completely withered leaves are sometimes torn away from the stem, and the scars left in these cases are probably a source of danger. In a few cases the cracks which form in the internodes of some varieties probably admit the fungus. One such case is recorded above. But the commonest points of entry in new infections of the above ground stem, in India, are undoubtedly the shoot and root eyes at the nodes.

The source of infection in new attacks.

We now come to another aspect of the subject and that is the source of infection in those cases in which new attacks occur in the cane stem. Practically all observers are agreed on the comparative rarity of the sporing stage on the surface of diseased cane stems, until these have dried up more or less completely. When we consider the extraordinarily abundant production of spores in most

fungi which depend on the wind for their dissemination and the chances of the individual spore alighting on a susceptible part of the cane stem, this point becomes of significance. But, as was pointed out in 1906, there is another part of the cane on which a fungus agreeing in morphological characters with *Colletotrichum falcatum* is frequently found and produces spores in greater quantity and more exposed to the wind than the stem form. This is the midrib of the leaf. Earlier writers have reported the occurrence of the fungus as a saprophyte on old, withered leaves of sugarcane.[1] That it also occurs not uncommonly as a leaf parasite seems to have escaped the notice of most observers, though Edgerton[2] refers to it. Experiments were carried out at Pusa to determine if the leaf form could infect the stem and conversely.

Three of these experiments have been described above (page 158) where it was shown, firstly, that wound inoculation with a pure culture of the midrib form caused visible infection of from one to three internodes after three months and, when the inoculated canes were planted, red rot developed in them with severity, and, secondly, that inoculation of the setts, immediately before planting, causes just as severe disease as when the stem form of the fungus is used. In another case a pure culture of the leaf fungus was used to inoculate Striped Mauritius cane, 11 inoculations being made towards the base and ten towards the apex of the stem. In a little over two months, 7 of the former were examined and showed 7+3+2+1+0+4+4 internodes affected. In 6 of the canes inoculated towards the apex, 3+1+0+1+1+2 internodes were found diseased after the same period. These experiments show that the leaf fungus can attack the stem and the cut setts, the symptoms produced being those of typical red rot.

The parasitism of the midrib form was next tested on leaves. In the first experiment spores from a pure culture were sown in drops of water on the upper surface of the uninjured midrib of growing canes. Out of 6 inoculations, none succeeded. The ex-

[1] Went, F. A. F. C. Notes on Sugarcane Diseases. Ann. of Bot. X, 1896, p. 588.
[2] Edgerton, *loc. cit.*, p. 4.

periment was repeated on 4 shoots in the laboratory, kept under bell jars to prevent the inoculated spot from drying rapidly. These also failed. In a third series, similar to the last, 6 inoculations were made without result. A month later, however, out of 12 similar inoculations, 5 succeeded. When the midrib was wounded, much better results were obtained. In the first trial 5 leaves were inoculated in plants growing in tubs in the laboratory, the upper epidermis being first removed by scraping with a sterile knife and the spot, after inoculation, being covered with damp sterile cotton wool to keep moist. All succeeded well, the characteristic red discoloration being well developed by the 9th day. In another series, 4 inoculations were made after injuring the epidermis of the midrib by touching it with a hot knife blade for 2 or 3 seconds. All took the infection severely. Four more were inoculated on another occasion, after scraping off the epidermis, and again all took.

Experiments were next made to test the parasitism of the stem form of *Colletotrichum falcatum* on the leaves. In the first experiment, spores from a pure culture were sown in drops of water on the upper surface of the uninjured midrib of canes growing in a tub in the laboratory. Of 15 inoculations, none succeeded. The experiment was again tried and of 7 inoculations, all succeeded. In a third series, out of 13 inoculations, 5 succeeded. When the midrib was wounded before inoculation the results were as follows. In the first trial 5 inoculations were made after scraping off the epidermis. The inoculated spot was covered with a pad of damp sterile cotton wool. None succeeded, the fungus growing by choice into the cotton wool. A similar experiment at a later date was made on 3 shoots standing in water, no cotton wool being used but the shoots being covered by bell jars. All succeeded. In a third experiment, the epidermis was injured by touching with a hot knife blade and all of 4 inoculations succeeded.

Though both leaf and stem forms, are capable of penetrating uninjured leaves, infection occurs much more readily when the leaf is wounded. The microscopic details of penetration will be described below. In nature, it has been observed that *Colletotri-*

chum is common around the hole which a minute boring insect frequently makes in the midrib. Salmon[1] has found in similar experiments with the mildews (*Erysiphaceæ*) that "green fly" (*Aphis*) has the same effect as a wound, in weakening the resistance of the plant cells to infection.

From these experiments it is apparent that there is no essential difference in the ability of the forms of *Colletotrichum*, found on the living midrib of the leaf and on the stem, to attack stems and leaves of sugarcane. Taken in conjunction with their morphological similarity, they must be held to be the same fungus. The species appears to be confined to sugarcane. The only other *Colletotrichum* resembling *C. falcatum* found widely distributed in India, is *C. Lineola* Corda, which attacks the leaves of jowar (*Andropogon Sorghum*) frequently. Morphologically the two species are closely allied, but the jowar fungus does not attack cane leaves. Out of 16 inoculations, half on unwounded spots on the midrib, half after scraping off the epidermis, none succeeded. Edgerton[2] failed to get symptoms of red rot by inoculating sugarcane stems with this species and also with the allied *C. cereale*. It is probable that many of the new attacks of red rot on the above-ground part of the cane stem, arise as a result of infection by spores blown from the diseased midribs of cane leaves. We do not see how it will ever be possible to avoid this, but it only gives greater force to the arguments in favour of concentrating attention on the elimination of diseased setts at the time of planting.

The actual penetration of the fungus into the tissues was studied in the leaf inoculations. Including both leaf and stem forms, altogether 63 inoculations were made on the uninjured midrib, of which 17 succeeded and 46 failed; while when the epidermis was wounded 20 out of 25 inoculations succeeded. In the successful cases where the epidermis was uninjured, penetration usually occurred not directly into the midrib but by superficial growth of the fungus until the large motor cells lying in groups on either side

[1] Salmon, E. S. Cultural experiments with "Biologic forms" of the *Erysiphaceæ*. Phil. Trans. Royal Society, Ser. B, Vol. 197, 1904, p. 112.

[2] Edgerton, C. W., *loc. cit.* p. 7.

of the midrib were reached; these were then penetrated (Fig. 1). As is already known, the germ-tubes of the spores readily form appressoria (described under the name of "gemmæ" by Went and of chlamydospores by most other writers). These are thick-walled, durable cells, capable of surviving detachment from the mycelium. It is probable that they serve a double purpose of close adhesion to the surface of the host plant and of accumulation of enzymic energy to secure penetration of its walls. In *Colletotrichum falcatum* the infection hypha seems to arise as a rule from an appressorium (Figs. 1 and 2). Entry through stomata was not observed, the infection hypha passing directly across the outer wall of an epidermal cell, or, in some cases, down between the side walls of two cells. After entry, the hyphæ may at once branch freely and fill the large motor cells with a mass of mycelium, or may penetrate deeply into the leaf, passing from cell to cell with ease in the large-celled parenchyma between the bundles, but not readily entering the latter. In some cases the sclerenchyma was penetrated, but usually, when the leaf had not been injured before inoculation, the hyphæ remained, for the first week at least, confined to the thin-walled cells. In the cases where the leaf was first wounded, conditions were somewhat different. There was a superficial growth as before, extending beyond the limits of the wound. But penetration was not now confined to the thin-walled motor cells but occurred freely into the epidermis of the midrib just beyond the wounded part (Fig. 2), and the mycelium ramified through the sclerenchyma as readily as through the parenchyma. In some cases, the layers of underlying sclerenchyma left after removing the epidermis, were an effective barrier and penetration only occurred beyond the midrib into the thinner tissues between the bundles, but in others, presumably when the injury was more severe, the thick-walled tissues showed much mycelium. All the invaded tissues developed a bright red colour.

In less than a week fructification may occur on the infected spot. The hyphæ first collect in masses in the epidermis, through

which they then break as stromatic bodies, on which the characteristic spores and sterile hairs are formed.

Relative susceptibility of the top and bottom of the cane.

Experiments were recorded in the previous Memoir[1] to show that much of the damage caused by red rot is due to inversion of the cane sugar. This appears to be because glucose is more readily assimilated by the fungus, growth in solutions in which the sugar was provided as glucose, being invariably better, at least at first, than when cane sugar was supplied. Flasks were prepared with solutions containing 10 per cent. cane sugar and glucose respectively together with peptone and sodium chloride, and inoculated with a loop of a suspension of spores, from a pure culture, in distilled water. The growth in the glucose flasks early took the lead and maintained its superiority for some weeks. More recently, Lewton-Brain[2] has dealt with the same question in considerable detail. He found that the inverting action of the fungus was considerable, but that, as stated in the previous Memoir, the actual consumption of sugar was small. This consumption, he found, fell entirely on the levulose. Thus in one experiment, though 75 per cent. of the sucrose was inverted, not one-twentieth part of this was actually consumed by the fungus and this appeared to be all levulose. The inversion was proved to be due to the presence of invertase, which was found both in the mycelium and also in the solution in which the fungus was grown.

Since it is known that the upper portion of the cane is richer in glucose, though poorer in total sugars, than the lower[3], experiments were made to compare the growth of the fungus in the top and bottom portions. In the first experiment, 3 sound Striped Mauritius canes were cut and brought to the laboratory. They were each inoculated at a lower and an upper internode by removing

[1] Butler, E. J., *loc. cit.*, 1906, p. 7.

[2] Lewton-Brain, L., *loc. cit.*, 1908, p. 32.

[3] Leather, J. W. Chemical composition of sugarcane and raw sugars. Agric. Ledger, No. 3 of 1897, p. 13.

a cylinder aseptically with a small cork borer and inserting a small quantity of a pure culture of *Colletotrichum*. After eight days they were examined. The lower inoculations were found to have infected 2+2+1 internodes respectively, the upper 1+2+4. The experiment was repeated with Samsara canes, 6 being inoculated in the same way. After seven days one was examined and was found to have 2 internodes infected at the base and 1 at the top. Two days later another was examined, 5 and 2 internodes respectively being found infected. Two days later the rest were examined. In one the infected portions had united in the middle. In the other three, 11+10+7 internodes were found infected at the base and 3+5+2 at the top. The experiment on page 167, where inoculations with the leaf form of the fungus were made at the top and bottom of growing canes, should also be compared. As the practical point at issue was to determine if any recommendations could be made for planting one part of the cane rather than another, where red rot is prevalent, the natural inversion that goes on after cutting was not taken into account, since it must equally go on in planted setts before germination. The experiments do not suggest that tops, though richer in glucose, can be more rapidly invaded by *Colletotrichum* than the rest of the cane and there appears to be no objection to their use from this point of view. The second experiment and that given on page 167, indeed suggest that the contrary is the case. Tops are also less likely to contain the fungus, when it has originated from below at a late stage in the growth of the cane.

Control of the disease.

The control of red rot was stated by the earlier investigators to be likely to be very difficult, owing to its position in the interior of the cane, the frequent absence of definite symptoms by which it might be detected in the growing crop and the practical impossibility of preventing wounds which would give an entry to the fungus. But of recent years little has been heard of the disease in Java, where it was first described, and it may be concluded that it has not proved so serious an enemy as was once feared.

In India it is in many places the greatest obstacle to successful cane cultivation. In Madras, Bombay and Bengal the area under thick cane has in certain districts periodically shrunk as a result of an accumulation of red rot in the crop, to expand again only when the diseased cane has been replaced from outside. Red rot has often been the limiting factor to the successful cultivation of heavy yielding canes.

The experiments given above are, we think, sufficient grounds for holding that this should not be the case; that, granted that a start is made with a healthy stock, it should be possible to keep the disease under control with no more than an occasional severe outbreak due to a specially unfavourable season.

The first requirement is to start with a healthy stock. In those districts, such as the Godavari Delta and some parts of Bombay Presidency,[1] where the local canes have become widely infected, new healthy seed must be brought in from outside. The very successful history of the Samalkota Sugar Station in the Godavari Delta, shows what excellent results may be obtained by this measure, under efficient supervision. It is highly probable that the little that has been heard of red rot in Java, in recent years, has been due to the efforts made to obtain good cane for planting, from special seed nurseries, combined with the growth of seedling canes which will be referred to below. As the Samalkota results are available in the Annual Reports of the Station, the methods adopted need not be more fully detailed. The past history of the cane must be taken into consideration. There is good evidence to show how dangerous it is to grow a variety from stock known to have been seriously infected, even though the crop may do well for the first few years. Large estates or groups of estates should be self-contained in the matter of seed supply and should possess a nursery or testing garden for the trial of new varieties under the best conditions. For the ordinary native cultivation, Government Farms should be utilized for the same purpose. In this way a supply of

[1] Kulkarni, G. S. Preliminary study of the red rot of sugarcane in the Bombay Presidency. Dept. of Agric., Bombay, Bull. No. 44, 1911, pp. 5-6.

healthy seed can be assured and new varieties can be introduced into cultivation as required.

Systematic and thorough selection of the setts used for planting must then be done each year or the new varieties will not maintain their freedom from disease for long. The methods to adopt are described in a previous paper[1] which should be referred to, and present no difficulties in practice. We consider that there is no single operation in the cultivation of thick canes in most parts of India of greater importance than this. It is not to be anticipated that the disease can be got rid of by a single selection nor, indeed, usually got rid of in its entirety by annual selection ; the most that is claimed is that it can be kept within reasonable limits in normal years and with good cultivation. The object of the selection is to prevent an accumulation of red rot to such an extent as materially to reduce profits and render it difficult to obtain a sufficient supply of sound seed for the coming season.

Of lesser importance, but still worth doing in most cases, is the regular removal of all withering clumps during the growing and ripening season. Such clumps, if left, dry up and produce spores, sometimes in considerable quantity. Infection of even perfectly sound cane through the aerial root eyes and through injured buds has been shown to occur and though our experience in Northern India has been that such infection is not common, it is perhaps more frequent in Madras, where the disease appears to be more virulent and rapid in its onset than with us. There is, also, the danger of infection through the soil, especially in irrigated cane, by means of the shed spores.

Judging from Godavari experience it is important to give cane a long rotation. How far this results from the peculiar circumstances of cane cultivation in heavy paddy soils, with very frequent irrigation, is not clear. In the Godavari Delta, the old practice was eight or nine years' rest from cane after a two years' cane crop (one year plant cane and one year ratoon). More recently, it has been reduced

[1] Butler, E. J., *loc. cit.*, 1907.

to six years, probably with bad results.[1] At Samalkota a two years' "dry" rotation (not followed by paddy) and a three years' "wet" rotation (followed by paddy) were tried, but found unsatisfactory. On the other hand, at Pusa, cane one year in five has been satisfactory and the non-irrigated cane in Bihar usually gets a much shorter rotation. The fungus appears to die out rapidly in moist soil, but cultures exposed to the air and kept moderately dry retain their vitality for at least five months. In Hawaii, Lewton-Brain found that plate cultures allowed to dry out invariably gave no sign of vitality after three months. But if cane is present, there seems to be little doubt that *Colletotrichum* lives and spreads through the soil and, in the young irrigated crop, passes from trench to trench, either with the seepage of irrigation water, on the feet and implements of coolies or (though less certainly) by direct growth through the soil. It has been shown that the roots are readily infected and we have lost several series of comparative experiments at Pusa through ground infection of control trenches. Fortunately the radius of spread does not appear to be large and if the measures detailed above are carried out, little injury should be caused in this manner.

In spite of all these precautions, serious attacks of red rot, from circumstances not ordinarily under control, may occur from time to time. We have met with two such cases. One was the outbreak at Pusa in 1907-08, which was, without doubt, the result of an epidemic of cane-fly (*Pyrilla aberrans*) on the cane that year. Leaf-hoppers are well known in other countries to be associated with bad attacks of fungus diseases as, for instance, in Hawaii, where "rind disease"[2] follows with great intensity the epidemics of leaf-hoppers. It is probable that the action of these insects is chiefly to reduce the vitality of the cane and render it increasingly sus-

[1] Wood, R. C. Scientific Report for the Samalkota Agricultural Station for 1908-09. Government Press, Madras, 1909.

[2] The cause of this disease is not yet quite clear. Howard in the West Indies and Went in Java held that it was identical with red rot, the fungus, *Melanconium Sacchari*, previously believed to be its cause, being merely a follower of *Colletotrichum falcatum*. Subsequent workers, such as Lewton-Brain, Cobb and Edgerton in the West Indies, Hawaii and Louisiana, have reverted to the previous view, though quite recently South and Dunlop have failed to establish the parasitism of *Melanconium Sacchari*. In the East, this fungus has not been recorded as a parasite.

ceptible to infection from aerial spores, which were formed in considerable quantity on the leaves during the outbreak at Pusa. The other case was a severe attack of red rot following extensive flooding of the cane fields, in some estates in Bihar several years ago. The effect of unfavourable external conditions such as this on the onset of the disease is discussed more fully below.

Susceptibility of varieties of cane.

Little light has been thrown by these investigations on the question of the relative susceptibility of different varieties of cane to red rot. One fact that is obvious to any observer of the disease in India is that the thin varieties of cane are, on the whole, less susceptible than the thick. Some of the Indian thin canes are so widely divergent from the thick races, that writers in Java have suggested that they may have originated from different species of *Saccharum*.[1] If so, one may perhaps anticipate that the relative immunity of the thin kinds will prove to be a deep seated "germ" character. With regard to the thick canes, certain observers, and in particular Dr. C. A. Barber, hold that the temporary or apparent immunity of certain thick varieties can be broken down by bad cultivation. He describes[2] how, in the Godavari Delta, successive canes have held favour during the past forty years, each in turn growing luxuriantly and bringing wealth, but after a few years becoming diseased. The constitution of each cane had been broken in turn by the ever-present fungus, until all the plants of that kind in the district were infected. Again he states[3] that of the varieties of cane brought from other countries for trial at Samalkota, none were really immune and it is probable that ultimately all will succumb in turn when placed under the adverse conditions of the local agriculture. In the same paper[4] he mentions as a curious fact that the Hospet cane varies greatly in its liability to disease in the different regions where it is found. A similar case occurred with the

[1] Kobus, J. D. Overzicht van het verloop der importatieplannen van vreemde rietsoorten op een eiland buiten Java. Archief v. d. Java-Suikerindustrie, II, 1894, p. 662.

[2] Barber, C. A. The Samalkota Sugarcane Farm. Agric. Journ. of India I, 1906, p. 45.

[3] *Ib.* Seedling canes in India. Agric. Journ. of India, VII, 1912, p. 324.

[4] *Ib. loc. cit.* p. 329.

Bombay Pundia cane which, when introduced at Samalkota, went out from disease in the first season, though the parent stock is little subject to red rot. Kulkarni[1] also notes the gradual deterioration of thick canes in parts of Bombay, and West Indian literature is full of references to the breaking down in health of the Bourbon cane, once widely grown, and this breaking down seems to have gone on more rapidly in some localities than in others.

These experiences with sugarcane are by no means unique amongst plants ordinarily propagated in a vegetative manner, that is by tubers, cuttings and the like. In Ireland the "Champion" potato was largely cultivated for many years, on account of its resistance to potato blight, but it has lost its resisting powers and been replaced by newer "seedling" varieties. Several similar cases are known, and the phenomenon is of considerable scientific interest.[2] Such progressive deterioration is in many cases apparently innate and has been likened to senescence, being capable of being checked when a new generation is started by sexual reproduction (*e.g.*, by raising from seed). But it can be hastened by exposure to unfavourable conditions or, on the other hand, be postponed by profound change in the environment. Barber states[3] that the surest way to induce red rot in cane to make its appearance is to plant the canes in a water-logged site. Harrison, in British Guiana, considers that the susceptibility of certain kinds of plants for instance, the Bourbon cane, to fungus attacks is due in part at least to defective soil conditions.[4] Such statements can be multiplied and must, we think, indicate a real phenomenon. In the opposite direction are such cases as that recorded by Calkins,[5] where a constantly changing culture medium was found to have the effect

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[1] Kulkarni, G. S. Preliminary study of the red rot of sugarcane in the Bombay Presidency. Department of Agriculture, Bombay, Bull. No. 44, 1911.

[2] c. f. Hartog, M. Problems of Life and Reproduction. Progressive Science Series, 1913, p. 19.

[3] Barber, C. A. The Samalkota Sugarcane Farm. Agric. Journ. of India, I, 1906, p. 45.

[4] Harrison, J. B. Varieties of Sugarcane and Manurial experiments in British Guiana. West Indian Bulletin, IX, 1909, p. 36.

[5] Calkins, Gary N. Protozoology, 1910, p. 109.

of prolonging the life of the race in Protozoa, bred from a single individual and not permitted to conjugate; when asexual propagation only is allowed and the environment kept constant, the race soon degenerates and dies out.[1]

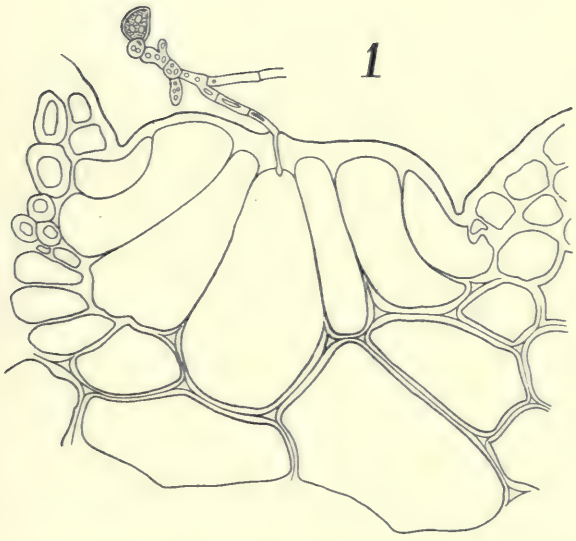
It is not unlikely, therefore that, there are two types of relative immunity, a genetic, such as is shown by the thin canes so widely cultivated in India, and a fluctuating, and that the latter is much more exposed to the influence of external conditions than the former. Indian thick canes, which have been subjected to vegetative propagation without a break for many generations, seem to show no evidence of genetic immunity. Hence frequent change of climate, good cultivation and good hygienic conditions generally, seem to be of great importance in preserving them from epidemics of disease.

The possession, by India, of a large range of relatively immune (to red rot) thin canes, of hardy habit and great tillering powers, though less productive than the thick canes of other countries, may prove an asset of the greatest value. The growth of seedling canes has been recently undertaken at the Coimbatore cane-breeding station under the control of Dr. Barber. If it is found possible by hybridization to combine the resistance of some of the thin canes to red rot, with the yielding qualities of a thick cane, a great step forward in enabling India to grow enough sugar for her own consumption and perhaps even to compete successfully with the sugar exporting countries, will have been taken. It is a happy augury that amongst the best of the canes now grown in Java (the most formidable competitor in sugar production that India has to meet) are the progeny of crosses between an Indian thin cane, the "Chunnee" obtained from Shahjahanpur (where, Dr. Barber informs us, it is locally called Chin), and the Cheribon thick cane grown in Java.

PUSA,
June 30*th*, 1913.

[1] Since this was written a very illuminating discussion of the deterioration of sugarcane varieties after long-continued vegetative propagation, written by Harrison, Stockdale and Ward (West Indian Bulletin, XIII, 1913, p. 177), has been received.

PLATE



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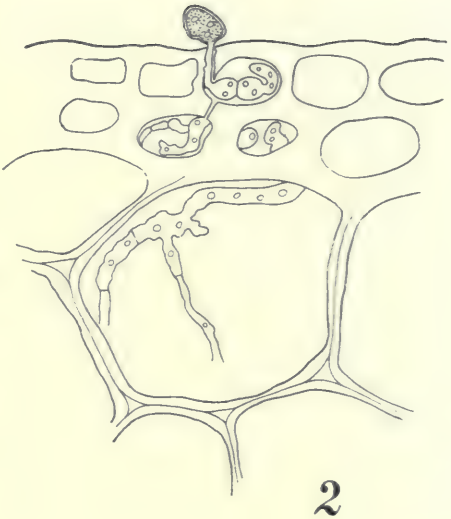

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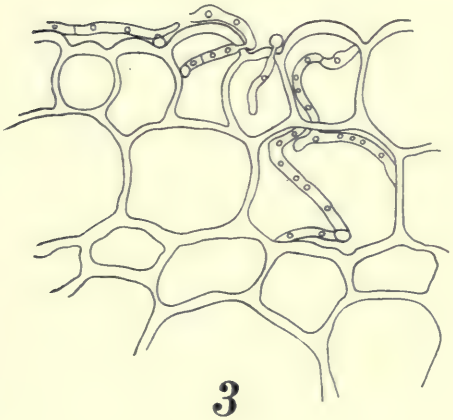

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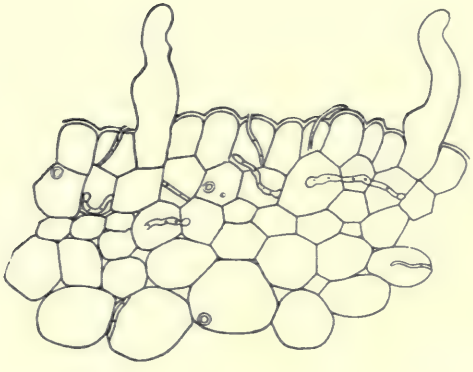

4

5

Infection of sugar cane leaves and roots by the red rot fungus, Colletotrichum falcatum.

DESCRIPTION OF THE PLATE.

Fig. 1. Penetration of a hypha, arising from an appressorium of the stem form of *Colletotrichum falcatum*, into a motor cell of the unwounded leaf of sugarcane. The midrib commences immediately on the right of the figure. X 350.

Fig. 2. Penetration of a hypha, arising from an appressorium of the stem form of *Colletotrichum falcatum*, into a cell of the thick-walled part of the epidermis at the edge of the midrib of the sugarcane leaf. The neighbouring central part of the midrib had been wounded by scraping off the outer layers with a sterile knife. X 500.

Fig. 3. Penetration of an adventitious root on the stem of sugarcane by hyphæ of *Colletotrichum falcatum*, through the uninjured epidermis. X 500.

Fig. 4. Penetration of an underground root of sugarcane by hyphæ of *Colletotrichum falcatum*, through the uninjured epidermis. X 300.

Fig. 5. Passage of the mycelium of *Colletotrichum falcatum* from a young inoculated adventitious root, of the same series from which fig. 3 was drawn, back to the main stem of the sugarcane. The hyphæ enter the vascular tissue and the parenchyma of the stem and spread in all directions. X about 50.

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